

THE TRANSPORT OF OXYGEN BY THE BLOOD OF FRESHWATER FISH

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INTRODUCTION

The stresses imposed by low oxygen pressures upon the ability of fish to oxygenate their blood and by low temperatures upon their ability to utilize oxygen in the blood were first appreciated by Krogh and Leitch (1919). In their study of four species of freshwater fish and two marine species they noted the apparent relationship between the pressure of oxygen required to oxygenate the blood and the pressure of oxygen in the external environment in which the species live. They stated that the difference in the properties of the blood (between fish living in poorly oxygenated water and well oxygenated water) was of such a magnitude as to suggest species differences. They also noted that carbon dioxide had a greater effect on the blood of fish than on mammalian blood in reducing the affinity of the hemoglobin for oxygen. This effect, known as the "Bohr effect" is thought to be an advantage in the transport of oxygen in that it should raise the pressure at which oxygen is discharged from the blood and thus should facilitate diffusion of oxygen to the tissues. The latter property, namely the enlarged Bohr effect, was considered by Krogh and Leitch as an adaptation to low environmental temperature, while the possession of a higher affinity to oxygen was regarded as an adaptation to low environmental oxygen tension. They suggested that these adaptations were made possible by special conditions within the erythrocyte affecting the hemoglobin.

Root (1931), in his careful examination of the blood of several marine teleosts, found that carbon dioxide reduced the maximal amount of oxygen combined, even in the presence of as high a pressure of oxygen as 150 mm. Hg. He found too that the blood of each species was very sensitive to carbon dioxide compared with mammalian blood, but his data do not indicate that the affinity of the hemoglobin varies significantly from species to species. Willmer (1934), in a study of the respiration of tropical freshwater fish, noted amongst other features that the blood varied in sensitivity toward carbon dioxide. He observed a relation between the environment of the fish and the sensi-

tivity of the blood towards carbon dioxide, the allegedly active river fish having the most sensitive blood and the fish of the swamp possessing the least sensitive blood. Powers *et al.* (1932, 1938) have presented some data on oxygen dissociation of freshwater fish blood and on the relation of environmental gases to blood gases. Black and Irving (1938) showed that the ability of carbon dioxide to alter the affinity of blood of certain fish depends largely upon the intact corpuscle.

The present paper deals with certain characteristics of the blood of four species of freshwater fish, which determine the capacity of the blood of the fish for the transport of oxygen. This has been accomplished by the construction of oxygen dissociation curves for whole blood at various pressures of carbon dioxide and at one temperature (15° C.). Comparison of the properties of the blood can only be made at the same temperature, and the temperature chosen was one at which all the species could survive with apparent ease. This allows the correlation of the properties of the blood with the environmental characteristics of the fish, but restricts deductions as to the natural functioning of the blood of each species in its characteristic environment whatever that may be.

The results obtained show specific differences in the affinity of the hemoglobin and, moreover, specific differences in the sensitivity of the whole blood to carbon dioxide, an effect which has been shown (Black and Irving, 1938) to depend in a large degree upon the intact erythrocyte. In addition, data are presented which show that blood of high oxygen affinity has a low sensitivity to carbon dioxide, and blood of low oxygen affinity possesses a high sensitivity to carbon dioxide.

MATERIALS AND METHODS

In this study four species of freshwater fish were used. These represented two superorders, Ganoidei and Teleostei of the subclass Actinopteri. The ganoid fish investigated was the bowfin, *Amia calva* Linnaeus. The teleost fish employed were the common sucker, *Catostomus commersonnii* (Lacépède), the carp, *Cyprinus carpio* Linnaeus and the catfish, *Ameiurus nebulosus* (Le Sueur).

The bowfin were obtained from the Great Lakes while the common suckers and carp were caught in Ontario lakes (Scugog and Erie). The catfish were provided by the Torresdale fish hatchery (Delaware river).

Blood was drawn from the heart by means of a Luer syringe. In the case of the three larger species, blood from a single fish was used for a number of curves. Dry heparin was added to prevent clotting. In no instance was any substance employed to prevent the normal respiration of the cells.

Immediately after the blood was drawn it was transferred to a tonometer of the type described by Irving and Black (1937). The gas mixtures were made up manometrically and the blood-gas mixture equilibrated in a water bath for not less than 20 minutes at 15° C. Emphasis should be placed on this fact, that only the drawn blood was equilibrated at 15° C. and not the fish. The common suckers and carp were taken from water, the temperature of which varied from 5° to 15° C.

The size of the sample of blood used for each analysis was 0.5 ml. Oxygen and CO₂ were determined by means of the "combined method" of Van Slyke and Neill. The reaction mixture was shaken in the manometric Van Slyke apparatus for at least 6 minutes, for oxygen is

TABLE I

The oxygen capacity and red cell volume of the blood of four freshwater fish

Species	Number of fish	Oxygen capacity vol. % HbO ₂	Red cell volume %	Oxygen capacity 100 ml. cells vol. % HbO ₂
Catfish, <i>Ameiurus nebulosus</i>	15			
Mean		13.3	32.2	41
Range		8.4-17.4	20.5-39.7	34-46
Carp, <i>Cyprinus carpio</i>	16			
Mean		12.5	33.1	37
Range		6.4-22.9	16.4-61.0	34-44
Bowfin, <i>Amia calva</i>	5			
Mean		11.8	27.1	44
Range		8.5-13.9	25.6-30.7	42-45
Common sucker, <i>Catostomus commersonnii</i>	39			
Mean		10.6	32.5	33
Range		5.9-14.6	20.4-48.5	21-43

often only slowly extracted from fish blood. The agreement between two consecutive determinations of oxygen capacity was within 0.3 volumes per cent. The gas phase was analyzed in a Haldane apparatus.

The factors used in the calculations were taken from tables given by Peters and Van Slyke (1932). In all cases the amount of physically dissolved oxygen was calculated using a solubility coefficient of 0.036 and subtracted so that the values given for oxygen represent the quantities combined with hemoglobin.

EXPERIMENTAL RESULTS

Oxygen Capacities and Red Cell Volumes

The upper limit of the quantity of oxygen which a given volume of blood can transport is determined by the oxygen capacity of the blood.

By oxygen capacity is meant the amount of oxygen (expressed as volumes per cent) combined with hemoglobin of whole blood when the pressure of oxygen is 153 mm. (that of air) and when the pressure of CO_2 , as shown by analysis of the gas phase, is less than 2 mm. Hg.

Individuals of all four species showed considerable variation in the oxygen capacity. The differences in the mean values for the four species as compared with the individual variations are small and hence the differences in the mean values are of no apparent significance. In comparing the oxygen capacities listed in Table I with the oxygen capacities of various animals compiled by Redfield (1933), the average value for the catfish, carp, bowfin and common sucker is higher than that for most marine fish but is well below the average for mammals.

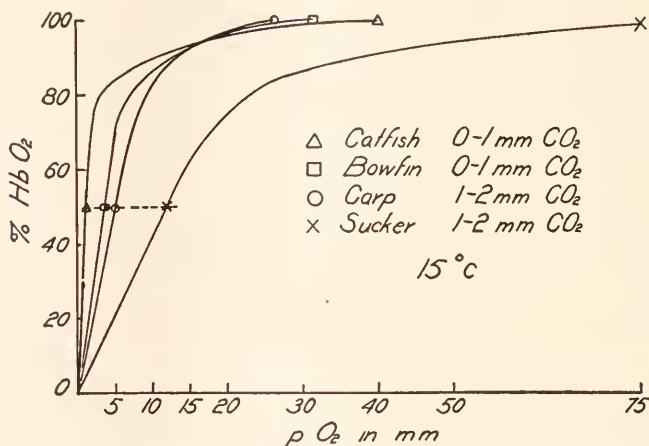


FIG. 1. Oxygen dissociation curves for the blood of the catfish, carp, bowfin and common sucker at $p\text{CO}_2$ 0-2 mm.

It is of some interest to note that the mean oxygen capacity for the bowfin, a ganoid, is about equal to the average of the bony fishes while it is twice that found for elasmobranchs by Dill, Edwards and Florkin (1932) and Ferguson, Horvath and Pappenheimer (1938).

Table I also gives the relation of oxygen capacity to red cell volume. Since red cell volume in any one sample of blood varied as a result of conditions not as yet completely investigated, little significance is attached to the values given.

The Combination of Oxygen with Blood

Oxygen Dissociation Curves in the Absence of CO_2 .—Because of the metabolism of the nucleated erythrocytes in fish blood it is difficult to obtain oxygen dissociation data in the complete absence of CO_2 . In-

asmuch as the effect of $p\text{CO}_2$ 2 mm. could not be discriminated from the effect of an undetectable pressure of CO_2 for the blood of the most sensitive fish, the oxygen dissociation curves for all the species at $p\text{CO}_2$ 0–2 mm. are considered to be in the virtual absence of CO_2 .

Oxygen dissociation curves for the blood of the four species are shown in Fig. 1. The data for the carp are taken from work published previously by Black and Irving (1937); the full data for the other three species are plotted in Fig. 2 (common sucker), Fig. 3 (catfish) and Fig. 4 (bowfin).

The curves for the four species seem to constitute one family of curves rising from the origin without evidence of an inflection which may, however, be present at higher temperatures.

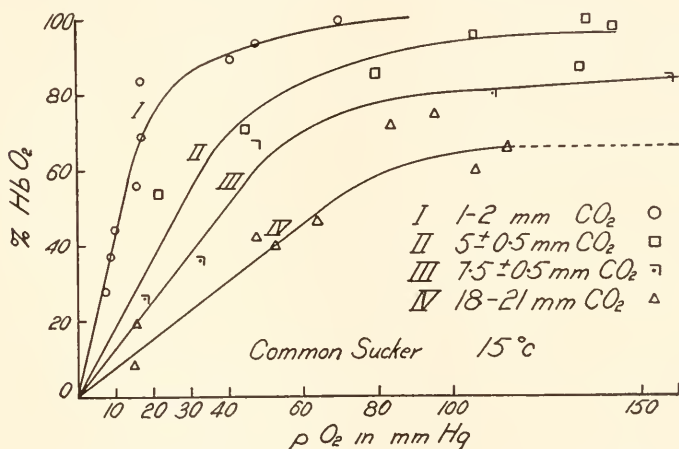


FIG. 2. Oxygen dissociation curves for the blood of the common sucker at various pressures of CO_2 . (N. B. Change in scale.)

The pressure of oxygen required to half saturate the blood is often taken as a characteristic of the dissociation curve. Krogh and Leitch (1919) called this the tension of unloading (t_u). This point does not necessarily represent the true conditions for unloading of fish blood so that the pressure at half saturation is used in this paper merely to represent the mid-point of the functional range of the hemoglobin. Further, the pressure of half saturation may be used as a single figure to represent the oxygen affinity of the blood, when the blood is equilibrated in the virtual absence of CO_2 . These pressures are given in Table II and reflect the fact that the curves for the catfish, bowfin and carp rise more steeply than that for the common sucker. They also indicate that, under these conditions of CO_2 and temperature, the

oxygen of the blood of the common sucker would be available to the tissues at higher pressures than in the other three fish.

Krogh and Leitch (1919) have also introduced the term "tension of loading" (t) to designate the pressure for 95 per cent saturation of

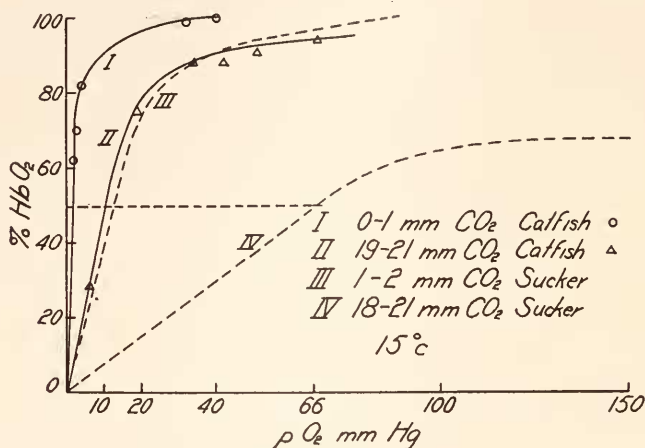


FIG. 3. Oxygen dissociation curves for the blood of the catfish and common sucker at pCO_2 0-2 and 20 mm.

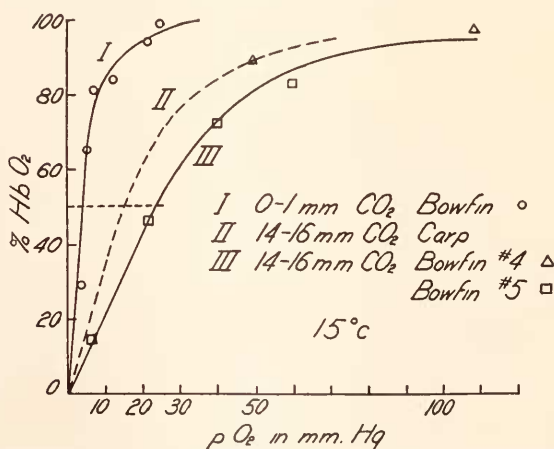


FIG. 4. Oxygen dissociation curves for the blood of the carp at pCO_2 15 mm. and for the blood of the bowfin at 1 and 15 mm. CO_2 .

the blood. This is a common degree of saturation for mammalian arterial blood but little satisfactory data exist to show the conditions of the blood coming from the gills of the fish. The loading tensions might also be used to designate the affinity of the hemoglobin for

oxygen and in fact they do in this series correspond qualitatively to the tension of unloading. They are not used here because they cannot be determined with any satisfactory degree of precision owing to the rapidly changing slope of the curves in this region.

The oxygen dissociation curves for blood *equilibrated in the absence of CO₂* are practically identical for the carp and the bowfin and they

TABLE II

Pressures of oxygen required for half saturation and loading of fish blood at various tensions of carbon dioxide and equilibrated at 15° C.

Species	pCO ₂	pO ₂ at 50% HbO ₂	pO ₂ at 95% HbO ₂
	mm. Hg	mm. Hg	mm. Hg
Catfish			
Sample 5 (single)	0-1	1.4	18
Sample 9 (4 pooled)	19-21	10	75
Sample 5 (single)	39	7.5	65
Sample 7 (2 pooled)	41	9	65
Sample 8 (2 pooled)	38	14	85
Carp			
(mean values of plotted data, Black and Irving, 1937)	1-2	5	17
	9-11	8	35
	14-16	14	70
	18-20	20	—
Bowfin			
Sample 4 (single)	0-1	4	19
Sample 5 (single)	12	11	
Sample 5 (single)	15	23	110
Common sucker			
(mean values of plotted data, Fig. 2)	1-2	12	53
	5	27	105
	7.5	40	—
	12*	52	—
	18-21	66	—

* Single point.

stand midway between the widely separated curves for the catfish and the common sucker (Fig. 1). From these data it may be said that the affinity for oxygen of the hemoglobin as it exists in the blood differs significantly in certain species of fish, a generalization first stated by Krogh and Leitch (1919). The marked differences found for the species mentioned above are in contrast to the similarity of the oxygen affinity of the blood of the five marine fish studied by Root (1931) and Green and Root (1933).

The Effect of CO₂ upon the Affinity for Oxygen of Whole Blood Studied

Common Sucker.—It is evident that CO₂ not only decreased the affinity for oxygen in the sense of increasing the pressure of oxygen required for half saturation, but also lowered the maximal amount of oxygen combined, *i.e.* at 150 mm. oxygen, as found by Root for certain marine teleosts. It has been demonstrated on carp and rainbow trout blood by Ferguson and Black (1940) that in the presence of CO₂ even a pressure of 700 mm. oxygen failed to restore the amount of oxygen combined with the hemoglobin to its former value.

It is apparent from Fig. 2 that increasing the pCO₂ from 2 to 20 mm. increases the oxygen pressure at half saturation by about 50 mm. The extreme individual variations found in seven fish give this mean figure a maximal spread of ± 6 mm. of oxygen. The magnitude of the sensitivity to CO₂ of the blood of the common sucker has been further confirmed on blood from nine other fish exposed to tensions of CO₂ of $5 \pm .5$ and $7.5 \pm .5$ mm.

Catfish.—The blood of the catfish showed the least sensitivity to CO₂ both as to decrease of maximal oxygen combined at high oxygen pressures and as to increased pressures of oxygen required at half saturation. Figure 3 contrasts the effect of CO₂ on catfish blood with mean curves of the blood of the common sucker.

The curve plotted for the blood of the catfish at pCO₂ 20 mm. represents the sample most sensitive to CO₂ but it shows an increase of only 8 mm. in the pressure of oxygen at half saturation and is in sharp contrast to the mean figure of 50 mm. for the common sucker. The difference between these species in sensitivity to CO₂ is far beyond the range of individual variation.

Carp and Bowfin.—Data for carp and bowfin are plotted in Fig. 4. The CO₂ sensitivities of the blood of these two species do not appear to differ greatly. The sensitivities are between those of the common sucker and the catfish but much closer to the latter. Very extensive studies would be necessary to establish a significant species difference between the carp and the bowfin though it may well exist.

DISCUSSION

Von Ledeboer (1939) cites Hall (1929) and Root (1931) and concludes that in certain instances, especially among marine fish, there seems to be a parallelism between the oxygen affinity of the hemoglobin and the activity of the species. However, Hall (1929) stated that in the three species studied the correlation was with the *amount* of hemoglobin per unit volume of blood, not with oxygen *affinity* of the hemoglobin. Root (1931) stated that "sluggish fish have bloods of low oxygen

capacity and the active of high capacity." Our data on oxygen capacities allow no deductions on this point since significant species differences were not found.

The differences in oxygen affinity are quite striking. An examination of Fig. 1 shows that the blood of the carp could be oxygenated to the extent of 90 per cent at a pressure of oxygen of 12 mm., at which pressure the blood of the common sucker is only half saturated. Again the remarkable blood of the catfish could serve at an environmental oxygen tension quite unsuited to the blood of the other species, for at pO_2 3 mm. its blood would be 80 per cent saturated while the blood of the carp and common sucker would only be 35 and 12 per cent oxygenated respectively.

Krogh and Leitch (1919) suggested a relation between the affinity of the blood for oxygen with the ability of the species to survive in environments low in oxygen. That this is an important relation is supported by the work of Fry and Black (1938), who have shown that the catfish, *A. nebulosus*, was able to remove oxygen at a lower tension than any of the other fish among the large series of fishes studied; as Wells (1913) found to be the case for a related species of catfish, *A. melas*.

The significance of the species difference in CO_2 sensitivity of the blood in relation to the ability of the blood to transport oxygen may be seen by considering the following hypothetical situations. If blood be fully oxygenated at the gills in the absence of CO_2 and transported to the tissues where metabolism proceeds until the pressure of both CO_2 and oxygen is 20 mm., then 25 per cent of the combined oxygen could be discharged from the blood of the catfish, 50 per cent from that of the carp and 85 per cent from the blood of the common sucker. Or viewed in another manner, the blood of the common sucker can be loaded (95 per cent HbO_2) at a pressure of 53 mm. Were this blood now exposed to the tissues where a pressure of 20 mm. CO_2 obtained, then 55 per cent (from 95 to 40 volumes per cent) of the combined oxygen could be utilized without any loss in the pressure of oxygen first required for loading. Under similar circumstances only about 15 per cent (from 95 to 80 volumes per cent) of the combined oxygen could be discharged by the blood of the catfish at its loading pressure of about 18 mm.

In terms of pressure of oxygen produced (at constant oxygenation) the specific differences in the ability of CO_2 to affect the equilibrium between oxygen and whole blood are no less interesting. If blood first oxygenated at the gills in the absence of CO_2 be transferred to the tissues where a tension of 20 mm. CO_2 exists, then when one-third

of the combined oxygen is used, the pressures of oxygen available to the tissues will be of the order of 15 mm. in the catfish, 30 mm. for the carp and 115 mm. for the common sucker.

It is an accepted concept of mammalian physiology that the Bohr effect contributes significantly to the efficiency of oxygen transport and might under certain circumstances be an essential factor in transportation of oxygen. Consequently it was thought that the wide differences in CO_2 sensitivity might reflect differences in habitual activity or capacity for maximal activity. Unfortunately, little data are available on the activity of fishes, habitual or maximal, in their natural environment or on metabolism, basal or maximal.

If CO_2 sensitivity of the blood is related to activity one would expect that the maximal activity of the catfish would be of a very low order. However, the maximal oxygen consumption of actively swimming catfish was found to be greater than the maximal oxygen utilization of the perch and common sunfish (Black, Fry and Scott, 1939). The blood of the perch is much more sensitive to CO_2 than the blood of the catfish (Irving, Black and Safford, 1939). It would seem then that no correlation between the CO_2 sensitivity of the blood and the rate of oxygen uptake of actively swimming fish is apparent. From this the inference is made that while the enlarged Bohr effect must be of importance to the efficiency of oxygen transport, each of the other factors such as circulatory efficiency, environmental temperature, the tension at which tissues can utilize oxygen, etc. is so important that the existence of a correlation between the activity of the fish and the single effect of CO_2 upon oxygen affinity of the hemoglobin may be masked. For example, J. S. Hart (1940) in his estimation of cardiac output for the catfish, carp, bowfin and common sucker found that the stroke volume of the catfish greatly exceeded the others while the heart of the common sucker had the smallest output per beat.

The possibility that the CO_2 effect upon the oxygen affinity may function as an oxygen pump is of the utmost interest in connection with the swimbladder. In their efforts to determine whether the mammalian lung secreted oxygen, Bohr and Haldane were greatly concerned with the mechanism for the deposition of gases in the swimbladder. An inspection of the oxygen dissociation curves of the blood of the common sucker at various pressures of CO_2 shows that the pressure of oxygen could be greatly increased by small pressures of CO_2 . These data extend the quantitative basis for the theory as put forth by Haldane (1922), Hall (1924) and Jacobs (1930). Of particular significance is the experimental demonstration (mentioned in results) that CO_2 will dissociate oxygen from hemoglobin at pressures up to 700 mm. Hg.

While the increased sensitivity to CO_2 should enhance the use of the blood in transporting oxygen and in making available a higher pressure of oxygen at unloading, this very sensitivity should hinder oxygenation at the gills. Fry and Black (1938) tested this implication and found that the common sucker with its CO_2 -sensitive blood was unable to remove oxygen from water containing CO_2 at tensions which did not hinder the respiration of catfish. Catfish proved to be the most resistant to the presence of CO_2 of all species studied, and it is a well-known fact that catfish are often found in swamps of acid water.

Although there is little evidence of significant differences in oxygen capacity, the experiments suggest strongly that the differences (a) in oxygen affinity of the blood and (b) sensitivity of the oxygen affinity to CO_2 , are important in natural respiration. Krogh and Leitch suggested that species variations might be considered as adaptations to environmental conditions, specifying particularly the degree of saturation of the water with oxygen. The experiments reported show that, in the catfish a high oxygen affinity is associated with low sensitivity to CO_2 while in the common sucker a low oxygen affinity is associated with a high CO_2 sensitivity. If specific differences in oxygen affinity constitute a basis for adaptation, then differences in CO_2 sensitivity of the blood must be considered as part of the adaptation of the blood. Krogh and Leitch recognized the importance of the CO_2 effect but did not observe that it differed greatly among the species.

The possession of blood characterized by a high affinity for oxygen would be expected to allow utilization of oxygen from water in which it was dissolved at a low tension. Such a hemoglobin, however, would impose the disadvantage that this oxygen would only be available to the tissues at a low pressure. This disadvantage might be offset by a high sensitivity to CO_2 whereby the CO_2 in the tissues would release the oxygen at a higher pressure. However, these experiments show that the catfish which has the highest oxygen affinity has no great CO_2 effect to offset it but indeed has the smallest CO_2 effect of the fish studied.

One other factor which might offset the apparent disadvantage of high oxygen affinity would be a higher environmental temperature, for higher temperature, like CO_2 , also tends to lower oxygen affinity or raise the pressure of unloading (t_u). It appears that the catfish in the summer frequents the shallower warmer water (Fry, 1939) and it is now apparent how the characteristics of the blood of the catfish should be more suitable in the warmer than in cooler strata of water.

The common sucker is found in deeper water in summer (Fry, 1939) where the possession of lower oxygen affinity and higher CO_2 sensitivity

offset the tendency of the lower temperature to decrease the pressure of unloading (t_u).

Experiments of Irving, Black and Safford (1940) (done at 15° C.) have shown that three species of trout (which are typically found in cold water), possess blood of even lower affinity for oxygen and greater sensitivity to CO₂ than that of the common sucker.

The low sensitivity to CO₂ and the high affinity for oxygen of the blood of the catfish are properties which could best be utilized for the transport of oxygen at higher rather than lower environmental temperatures. Conversely a low oxygen affinity and a high CO₂ sensitivity of the blood (such as characterize that of the common sucker or the trout) are properties which would tend to offset the lowered temperature effect in the transport of oxygen by the blood. The separate adaptive features of lowered oxygen affinity and enlarged Bohr effect of fish blood in an environment of low temperature were set forth by Krogh and Leitch (1919). However, their data did not show that high affinity for oxygen is in general possessed by blood of low CO₂ sensitivity and that blood of low affinity for oxygen has a large CO₂ effect. Moreover, their data did not show that species differences existed in the sensitivity of the blood to CO₂. That gradations in the CO₂ effect may be of importance in the economy of fish inhabiting different thermal strata of freshwater lakes has been discussed very clearly by Fry (1939).

Krogh and Leitch suggested that the specific characteristics of the hemoglobin in the different bloods must be due to specific conditions in the different erythrocytes. This view was based on the belief common at that time that the hemoglobin was identical in all species. It is now known that considerable differences in the properties can be found due to differences in the globin. It remains to be shown to what extent the specific characteristics of these bloods are due to different hemoglobins and to what extent due to special conditions in the erythrocytes. Hall and McCutcheon (1938) have made an extensive examination of the differences in the hemoglobins of marine fish. It seems important to point out that these differences in hemoglobins do not always explain the sum total of the differences between whole bloods as regards oxygen transport, for Black and Irving (1938) have shown for the blood of the carp, bowfin and common sucker that when hemoglobin is released by hemolysis the effects of CO₂ on oxygen affinity become so strikingly different that it is at present impossible to predict the properties of the hemoglobin within the red cell from its observed properties in solution. The explanation given by Krogh and Leitch for the basis of adaptation in the blood of fish

may then be extended, for the specificity of the oxygen affinity of the hemoglobin and the sensitivity of the hemoglobin to CO_2 as it exists in fish blood are undoubtedly a combination of the two factors, specificity of the hemoglobin moiety and the specific influence of the intact corpuscle.

SUMMARY

1. Oxygen capacities have been determined on the blood of four freshwater fish (catfish, carp, bowfin and common sucker). Individual variations are larger than average interspecific differences.

2. Significant differences between species were found for the affinity of the hemoglobin in whole blood for oxygen in the absence of CO_2 and at 15°C . in these freshwater fish as contrasted with the rather uniform oxygen affinity of most marine fish studied so far.

3. Significant differences between species were found for the effect of CO_2 on the affinity of the hemoglobin in whole blood for oxygen. For example, at 15°C . an increase in $p\text{CO}_2$ from 2 to 20 mm. causes an increase in oxygen pressure at half saturation of 8 mm. for the catfish and of 50 mm. for the common sucker.

4. Blood with high oxygen affinity has a low sensitivity to CO_2 (catfish). Blood with low affinity for oxygen has a high CO_2 sensitivity (common sucker).

5. It is suggested that since blood characterized by high oxygen affinity lacks a large CO_2 effect which could offset the disadvantage imposed at deoxygenation, the blood would function best in a high environmental temperature.

6. The greater sensitivity to CO_2 of fish blood as compared with mammalian blood was regarded by Krogh and Leitch as an adaptation to lower temperature. Evidence can now be presented to show that freshwater fish of higher CO_2 sensitivities are found in the colder habitats.

7. The very striking effects of CO_2 show that high pressures of oxygen might be developed within the fish by the presence of relatively low pressures of CO_2 . The old suggestion that the Bohr effect may be responsible for the high percentage of oxygen in the swimbladder of many fish is thus given additional quantitative support.

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LITERATURE CITED

- BLACK, E. C., AND LAURENCE IRVING, 1937. The effect of carbon dioxide upon the oxygen capacity of the carp (*Cyprinus carpio* L.). *Trans. Roy. Soc. Canada*, Section V: 29-32.
- BLACK, E. C., AND LAURENCE IRVING, 1938. The effect of hemolysis upon the affinity of fish blood for oxygen. *Jour. Cell. and Comp. Physiol.*, **12**: 255-262.
- BLACK, E. C., F. E. J. FRY AND W. J. SCOTT, 1939. Maximum rates of oxygen transport for certain freshwater fish. *Anat. Rec.*, **75**: (Supplement) 80.
- DILL, D. B., H. T. EDWARDS AND M. FLORKIN, 1932. Properties of the blood of the skate (*Raia oscillata*). *Biol. Bull.*, **62**: 23-36.
- FERGUSON, J. K. W., AND EDGAR C. BLACK, 1940. Unpublished data.
- FERGUSON, J. K. W., S. M. HORVATH AND J. R. PAPPENHEIMER, 1938. The transport of carbon dioxide by erythrocytes and plasma in dogfish blood. *Biol. Bull.*, **75**: 381-388.
- FRY, F. E. J., 1939. The position of fish and other higher animals in the economy of lakes. *The Am. Assoc. Adv. Sci.*, Publ. No. 10: 132-142.
- FRY, F. E. J., AND E. C. BLACK, 1938. The influence of carbon dioxide on the utilization of oxygen by certain species of fish in Algonquin Park, Ontario. *Anat. Rec.*, **72** (Supplement): 47.
- GREEN, A. A., AND R. W. ROOT, 1933. The equilibrium between hemoglobin and oxygen in the blood of certain fishes. *Biol. Bull.*, **64**: 383-404.
- HALDANE, J. S., 1922. *Respiration*. New Haven.
- HALL, F. G., 1924. The functions of the swimbladder of fishes. *Biol. Bull.*, **47**: 79-126.
- HALL, F. G., 1929. The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Am. Jour. Physiol.*, **88**: 212-218.
- HALL, F. G., AND F. H. MCCUTCHEON, 1938. The affinity of hemoglobin for oxygen in marine fishes. *Jour. Cell. and Comp. Physiol.*, **11**: 205-212.
- HART, J. S., 1940. Personal communication.
- IRVING, LAURENCE, AND E. C. BLACK, 1937. A convenient type of tonometer for the equilibration of blood. *Jour. Biol. Chem.*, **118**: 337-340.
- IRVING, LAURENCE, E. C. BLACK AND V. SAFFORD, 1939. The respiratory tolerance of some Pennsylvania fish. *Am. Jour. Physiol.*, **126**: P545-546.
- IRVING, LAURENCE, E. C. BLACK AND V. SAFFORD, 1940. The effect of temperature and CO₂ upon the oxygenation of fish blood. *Am. Jour. Physiol.*, **129**: 387-388.
- JACOBS, W., 1930. Untersuchungen zur Physiologie der Schwimmblase der Fische. *Zeitschr. f. vergl. Physiol.*, **11**: 565-629.
- KROGH, A., AND I. LEITCH, 1919. The respiratory function of the blood in fishes. *Jour. Physiol.*, **52**: 288-300.
- VON LEDEBUR, JOACHIM, 1939. Der Sauerstoff als ökologischer Faktor. *Erg. d. Biol.*, **16**: 173-261.
- PETERS, JOHN P., AND DONALD D. VAN SLYKE, 1932. *Quantitative Clinical Chemistry*. Volume II, Methods. Baltimore.
- POWERS, EDWIN B., ET AL., 1932. The relation of respiration of fishes to environment. *Ecol. Mon.*, **2**: 385-473.

- POWERS, EDWIN B., S. M. WINGO AND L. M. SHIPE, 1938. The relation of respiration of fishes to environment. XIII. Notes on the effect of the carbon dioxide tension of the water on the hemoglobin of the blood and upon the composition of the swimbladder gas. *Tenn. Acad. Sci.*, **13**: 21-25.
- REDFIELD, ALFRED C., 1933. The evolution of the respiratory function of the blood. *Quart. Rev. Biol.*, **8**: 31-57.
- ROOT, R. W., 1931. The respiratory function of the blood of marine fishes. *Biol. Bull.*, **61**: 427-456.
- WELLS, M. M., 1913. Resistance of fishes to different concentrations and combinations of oxygen and carbon dioxide. *Biol. Bull.*, **25**: 323-347.
- WILLMER, E. N., 1934. Some observations on the respiration of certain tropical freshwater fishes. *Jour. Exp. Biol.*, **11**: 283-306.